IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of: | Confirmation No.: 8131 |
| Chang *et al.* | Art Unit: 1642 |
| Appl. No. 10/765,568 | Examiner: Halvorson, M. M. |
| Filed: January 28, 2004 | Atty. Docket: 2474.0100001/BJD/JKM/TCS |
| For: Method For Evaluating The Efficacy Of Certain Cancer Treatments | |

**Declaration of Kathleen F. Pirollo Under 37 C.F.R. § 1.131(a)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Kathleen F. Pirollo, hereby declare and state as follows:

2. I am one of the three named inventors of U.S. Application No. 10/765,568, filed January 28, 2004, entitled *Method For Evaluating The Efficacy Of Certain Cancer Treatments*. This declaration is made in support of the Amendment and Reply filed herewith.

3. I hold the degree of Doctor of Philosophy. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

4. Attached hereto as Exhibit B are photograph reproductions of two western blots that were produced by me and/or under my direction.

5. Exhibit B shows the results of experiments designed to detect apoptosis in breast cancer patients. In panel A, a series of serum samples obtained from breast cancer patients were not purified but were run directly on a NuPAGE Novex Bis-Tris 4-12%

gradient gel (Invitrogen). Prior to loading the samples, 5uL of each serum was mixed with 6.25uL NuPAGE LDS sample buffer (4X), 2.5 uL NuPAGE reducing agent (10X) and 11.25uL of deionized water to bring the volume up to 25uL and the mixture heated at 70°C for 10 minutes. The entire 25uL sample was subsequently loaded onto the gel which was run using the buffers recommended by the manufacturer at 140 V for approximately 2 hours until the 9kDa band of the protein standard (Fermentas PageRuler™ prestained protein ladder) is at the bottom of the gel. The proteins on the gel were then transferred to PROTRAN® nitrocellulose membrane (0.2um pose size) (Schleicher & Schuell) using standard techniques and the membrane was probed, using standard procedures, with rabbit polyclonal Cleaved Caspase 3 (Asp175) Antibody (Cell Signaling Technology) which detects the 17/19kDa fragment of activated caspase 3, but does not recognize full length caspase-3 or other cleaved caspases. Goat anti-rabbit (Jackson Immunoresearch Labs) was used as the secondary antibody. Signal was detected using the ECL™ Western Blotting Detection kit (Amersham/GE Healthcare) according to the maufacturer's protocol.

As shown in Panel A, the location where the 17kDa fragment of caspease 3 would be present is obscured by non-specific signal generated by the presence of porphyrins in the serum. Porphyrins are naturally occurring compounds found in hemoglobin, myoglobin and cytochromes. All serum and plasma samples obtained from whole blood contain porphyrins at varying levels due to breakdown of hemoglobin in the whole blood and thus are present in whole blood and whole blood products.

In contrast to panel A, the amount of the17kDa fragment is easily detectable in the human serum samples in panel B, all of which were purified and run according to the methods described in this application in the Examples (e.g. Examples 2, 3, 4 and 14) using

the MICRO-BIO-SPIN® P6 chromatography column. In panel B, the Western blot was performed as described for panel A. Lanes A and B in panel B are the identical serum samples used pre-purification in lanes A and B above in panel A.

6. I further state that all statements made on my own knowledge are true and that all statements made on information and belief are believed to be true and further that willful false statements and the like are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the U.S. Code and may jeopardize the validity of the application or any patent issuing thereon.

10/3/07
Date

Kathleen F. Pirollo

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# EXHIBIT A

EXHIBIT A
Appl. No. 10/765,568

# CURRICULUM VITAE

## I. PERSONAL


Name: Kathleen Frances Pirollo

Date of Birth: August 19, 1951

Place of Birth: Philadelphia, PA., USA

Citizenship: U.S.

Marital Status: Not Married

Work Address: Department of Oncology
Georgetown University Medical Center
Lombardi Cancer Center/TRB E420
3970 Reservoir Road NW
Washington, DC 20007-2197
Phone: (202) 687-8418
FAX: (202) 687-8434

Home Address: 547 Anderson Ave
Rockville, MD 20850
(301) 610-9356


## II. EDUCATION

1. 1975 - 1982 Hahnemann University, Philadelphia, PA. Ph.D., Biochemistry
   1973 - 1975 Philadelphia College of Pharmacy and Science, Philadelphia, PA. M.S., Pharmaceutical Chemistry
   1969 - 1973 Philadelphia College of Pharmacy and Science, Philadelphia, PA. B.S., Chemistry

2. Postdoctoral Training

   1982 - 1985 Postdoctoral Fellow, The Wistar Institute, Philadelphia, PA.

3. Other Study and Research Opportunities

   1985 - 1988 Research Associate, Pathology Department, USUHS, Bethesda, MD.
   1973 - 1975 Teaching Assistant, Philadelphia College of Pharmacy and Science

## III. PROFESSIONAL EXPERIENCE

| | | |
|---|---|---|
| 1. | 1998 – Present | Research Associate Professor, Department of Otolaryngology, Lombardi Cancer Center, Georgetown University, Washington, D.C. |
| 2. | 1996 - 1998 | Research Assistant Professor, Department of Otolaryngology, Lombardi Cancer Center, Georgetown University, Washington, D.C. |
| 2. | 1994 - 1996 | Senior Research Associate, Department of Surgery, Division of OHNS, Stanford University, Stanford, CA. |
| 3. | 1993 - 1994 | Research Associate Professor, Dept. of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD. |
| 4. | 1988 - 1993 | Research Assistant Professor, Dept. of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD. |

## IV. HONORS AND AWARDS

Awards:

1973 - National Institute of Chemists Award
1970 - William L. Cliffe Scholarship
1969 - Henry Bower Memorial Scholarship

## V. MEMBERSHIPS IN PROFESSIONAL SOCIETIES

American Association for Cancer Research
American Society of Gene Therapy

## VIII. TEACHING ACTIVITIES

1. Lecturer in 1st year Medical School course "Biochemistry" at Georgetown University.
2. Lecturer in 2nd year Medical School course "Genetics", at Georgetown University.
3. Lecturer in Graduate Level Course "Principles and Practice of Molecular Therapy", at Georgetown University.
4. Lecturer in Graduate Level Course "Recombinant DNA Technology and Applications" at Uniformed Services University of the Health Sciences.
4. Laboratory instructor for DNA Probes portion of Pathology course at the Uniformed Services University of the Health Sciences.

## IX. SCHOLARSHIP AND RESEARCH

### A. RESEARCH GRANTS

*Previously Funded*

1. Principal Investigator of USUHS grant RO74DK, "Association of Radioresistance with a Cancer-prone family." April 1, 1990 to March 31, 1993. 40% effort.
2. Co-Principal Investigator on NIH grant RO1CA45158, "Oncogenes in Human Cancer Induction." Dec. 1, 1989 to Nov. 30, 1994. 50% effort.

3. Co-Principal Investigator on NIH Competitive Renewal of RO1CA45158, "Status of P53 in a LFS Cancer-Prone Family." Dec. 1, 1994 to Nov. 30, 1998. 50% effort.
4. Principal Investigator on Pilot Project under NIH SPORE Grant P50 CA58185, "Sensitization of Breast Cancer to Chemotherapy by Systemic Delivery of Anti-HER-2 Oligonucleotides." Sept. 1, 1997 to August 31, 1998.
5. Principal Investigator on grant from SynerGene Therapeutics, Inc., "Evaluation of a Systemic Gene Delivery System *In Vitro* and *In Vivo*" April 1,.1998 to March 31, 2000.

*Pending*

6. Principal Investigator of Georgetown University component of an STTR Phase I application in conjunction with SynerGene Therapeutics, Inc. "Targeting Stealth Liposome for Cancer Gene Therapy" (1R41-CA 91660-01) June 1, 2002 to May 31, 2003.
7. Principal Investigator of Georgetown University component of an STTR Phase II application in conjunction with SynerGene Therapeutics, Inc. "Immunoliposome- Mediated Gene Therapy for Prostate Cancer" (2R42CA80449-02). Sept. 1, 2002 to August 31, 2004.
8. Principal Investigator of the Georgetown University components of STTR Phase I and Phase II grants in conjunction with SynerGene Therapeutics, Inc entitled "A Dual Molecular/ Targeting therapy for PanCA (1R41 CA 103549-01 and 1R42 CA 103549-01) September 1, 2003 to August 31, 2006.
9. Principal Investigator of the Georgetown University component of an STTR Phase I grant in conjunction with SynerGene Therapeutics INC entitled "Enhancement of Tumor-Targeted Trangene Expression" (1R41 CA 105581-01) Sept. 1, 2004 to August 31, 2005.

## B. PUBLICATIONS

### 1. ORIGINAL PAPERS IN REFEREED JOURNALS

1. Soslau, F.G., and **PIROLLO, K.F.** Selective Inhibition of Restriction Endonuclease Cleavage by DNA Intercalators. BBRC. 115:484-491, 1983.
2. **PIROLLO, K.F.**, Avdalovic, N., and Diamond, L. Transforming Genes from Benzo(A)pyrene-transformed Syrian Hamster cell lines. J. Biochemical and Molecular Epidemiology of Cancer. 1985 UCLA Symposia.
3. Chang, E.H., Morgan, P.L., Lee, E.J., **PIROLLO, K.F.**, White, E.A., Tsichlis, P.N., and Patrick, D.H.: Pathogenicity of Retroviruses Containing Either the Normal c-Ha-ras-1 Gene or its Mutated Form Derived From the Bladder Carcinoma EJ/T24 cell line. J. Exper. Path. 2:177-190, 1985.
4. Chang, E.H., **PIROLLO, K.F.**, Zou, Z.Q., Cheung, H.Y., Lawler, E.L., Garner, R., White, E.A., Bernstein, W.B., Fraumeni, J.Jr., and Blattner, W.A. Oncogenes in Radioresistant, Non-cancerous Skin Fibroblasts From a Cancer-prone Family. Science 237:1036-1039, 1987.
5. Bernstein, W.B., Zou, Z.Q., Black, R.A., **PIROLLO, K.F.** and Chang, E.H. Association of γ-Interferon Induced Growth Inhibition and Modulation of Epidermal Growth Factor Receptor Gene Expression in Squamous Cell Carcinoma Cell Lines. J. Biol. Regulators Hemeo. Agents. 2:186-192, 1988.
6. **PIROLLO, K.F.**, Garner, R., Yuan, S-Y., Li, L, Blattner, W.A., and Chang, E.H. RAF Involvement in the Simultaneous Genetic Transfer of the Radioresistant and Transforming Phenotypes. Inter. J. of Rad. Biol. 55:783-796, 1989.

7. Cunningham, J.M., Francis, G.E., Holland, M.J., **PIROLLO, K.F.** and Chang, E.H. Aberrant DNA Topiosomerase II Activity, Radioresistance, and Inherited Susceptibility to Cancer. Br. J. Cancer 63: 29-36, 1991.
8. Srivastava, S., Zou, Z., **PIROLLO, K.F.**, Blattner, W., and Chang, E.H. Germ-line Transmission of a Mutated p53 Gene in a Cancer-prone Family with Li-Fraumeni Syndrome. Nature 348:747-9, 1991.
9. Chang, E.H., Miller, P.S., Cushman, C., Devadas, K., **PIROLLO, K.F.**, Ts'o, P.O.P., and Yu, Z.P. Antisense Inhibition of *ras* p21 Expression that is Sensitive to a Point Mutation. Biochemistry 30:8283-8286, 1991.
10. McDaniel, T., Carbone, D., Takahashi, T., Chumakou, P., Chang, E.H., **PIROLLO, K.F.**, Ying, J., Huang, Y., and Meltzer, S.J. The MSP I Polymorphism in Intron 6 of p53 (TP53) Detected by Digestion of PCR Product. Nucleic Acids Research 19(17):4796, 1991.
11. Srivastava, S., Tong, Y.A., Devadas, K., Zou, Z.-Q., Sykes, V.W., Chen, Y., Blattner, W.A., **PIROLLO, K.F.** and Chang, E.H. Detection of both Mutant and Wild-Type P53 Protein in Normal Skin Fibroblasts and Demonstration of a Shared "Second Hit" on P53 in Diverse Tumors form a Cancer-Prone Family with Li-Fraumeni Syndrome. Oncogene 7:987-991, 1992.
12. Srivastava, S.K.,, Tong, Y.A., Devadas, K., Zou, Z.-Q., Chen, Y., **PIROLLO, K.F.**, Chang, E.H. The Status of the p53 Gene in Human Papilloma Virus Positive or Negative Cervical Carcinoma Cell Lines. Carcinogenesis, 13:1273-1275, 1992
13. Kasid, U., **PIROLLO, K.F.**, Dritschilo, A., and Chang, E.H. Oncogenic Basis of Radiation Resistance. Advances in Cancer Research 61:195-233, 1993.
14. Srivastava, S., Wang, S., Tong, Y.A., **PIROLLO, K.F.** and Chang, E.H. Several Mutant p53 Proteins Detected in Cancer-Prone Families with Li-Fraumeni Syndrome Exhibit Transdominant Effect on the Biochemical Properties of the Wild-Type p53. Oncogene 8:2449-2456, 1993.
15. **PIROLLO, K.F.**, Tong, Y.A., Villegas, Z., Chen, Y., Chang, E.H. Oncogene Transformed NIH3T3 Cells Display Radiation Resistance Levels Indicative of a Signal Transduction Pathway Leading to the Radiation Resistant Phenotype. Radiation Research 135:234-243, 1993.
16. Parshad, R., Price, F.M., **PIROLLO, K.F.**, Chang, E.H., and Sanford, K.K. Cytogenic Response to $G_2$ Phase X-irradiation in Relation to DNA Repair and Radiosensitivity in a Cancer-Prone Family with Li-Fraumeni Syndrome. Radiation Research, 136:236-240, 1993.
17. Janat, M.F., Srivastava, S., Devadas, K., Chin, G.A., **PIROLLO, K.F.**, and Chang, E.H. Inhibition of the Retinoblastoma (RB) Protein Phosphorylation by the Synergistic Effect of Interferon-$\gamma$ and Tumor Necrosis Factor-$\alpha$. Mol. Cel. Differen. 2(3): 241-253, 1994.
18. **PIROLLO, K.F.**, Hao, ZM., Rait, A., Jang, Y-J, Fee Jr., W.E. Ryan, P., Chaing, Y., and Chang, E.H. P53 Mediated Sensitization of Squamous Cell Carcinoma of the Head and Neck to Radiotherapy. Oncogene, 14, 1735-1747, 1997.
19. Chang, E.H., Hao, ZM., Rait, A., Jang, Y-J, Fee Jr., W.E., Sussman, H.H., Murphy, G., Ryan, P., Chiang, Y., and **PIROLLO, K.F.** Restoration of the G1 Checkpoint and the Apoptotic Pathway mediated by Wild-Type p53 Sensitizes Squamous Cell Carcinoma of the Head and Neck to Radiotherapy. Archives of Otolaryngology Head and Neck Surgery, 123: 507-512, 1997.
20. Xu, L., **PIROLLO, K.F.**, and Chang, E.H. Transferrin-Liposome-Mediated p53 Sensitization of Squamous Cell Carcinoma of the Head and neck to Radiation *In Vitro*. Human Gene Therapy, 8, 467-475, 1997.
21. **PIROLLO, K.F.**, Rait, A., Ho, C.W., and Chang, E.H. Evidence Supporting a Signal

Transduction Pathway Leading to the Radiation-Resistant Phenotype in Human Tumor Cells. Biochemical and Biophysical Research Communications, 230, 196-201, 1997.

22. Xu, L., **PIROLLO, K.F.**, Rait, A., Murray, A.L., and Chang, E.H. Systemic p53 Gene Therapy In Combination with Radiation Results in Human Tumor Regression. Tumor Targeting, 4: 92-114, 1999.
23. Rait, A., Krygier, J.E., **PIROLLO, K.F.**, and Chang, E.H. Sensitization of Breast Cancer Cells to Taxol by Antisense HER-2 Oligonucleotides. Antisense & Nuc. Acid Drug Dev, 9: 403-408, 1999.
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27. Chang, E.H., **PIROLLO, K.F.**, and Bouker, K.B. p53 Gene Therapy: Key to Modulating Resistance to AntiCancer Therapies. Molecular Medicine Today, 6: 358-364, 2000.
28. **PIROLLO, K.F.**, Bouker, K.B., and Chang, E.H. Does p53 Status Influence Tumor Response to Anti-Cancer Therapies. Anti-Cancer Drugs, 11: 419-432, 2000.
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30 Rait, A., Rait, V., **PIROLLO, K.F.**, and Chang, E.H. Inhibitory Effects of the Combination of HER Antisense Oligonucleotide and Chemotherapeutic Agents Used for Treatment of Human Breast Cancer. Cancer Gene Therapy, 8:728-739, 2001.
31 Sherif, Z.A., Nakai, S., **PIROLLO, K.F.**, Rait, A., and Chang, E.H. Down-modulation of bFGF-Binding Protein Expression Following Restoration of p53 Function-A Possible Mechanism for the Bystander Effect. Cancer Gene Therapy, 8:771-781, 2001.
32 Xu, L., Tang, W., Huang, C., Alexander, W., Yang, Q., Xiang, L., **PIROLLO, K.F.,**, Rait, A., and Chang., E.H. Systemic p53 Gene Therapy of Cancer with Immuno-lipoplexes Targeted by Anti-Transferrin Receptor scFV. Molecular Medicine, 7(10): 723-734, 2001.
33 Xu., L, **PIROLLO, K.F.**, Rait, A., Xiang, L., Cruz, M., Huang, W-Q, and Chang, E.H. Systemic Tumor-targeted Gene Delivery by Anti-transferrin Receptor scFV-Immunoliposomes. Molecular Cancer Therapeutics. 1: 337-346, 2002.
34 Xu, L., Frederick, P., **PIROLLO, K.F.**, Tong, W-H., Rait, A., Xiang, L-M., Huang, W., Cruz, I., Yin, Y., and Chang, E.H. Self-Assembly of a Virus-mimicking Nanostructure System for Efficient Tumor-targeted Gene Delivery. Human Gene Therapy. 13:469-481, 2002.
35 Rait, A.S., **PIROLLO, K.F.**, Xiang, L-M., Ulick, D., and Chang, E.H. Tumor Targeting, Systemically Delivered Antisense HER-2 Chemosensitizer Human Breast Cancer Xenografts Irrespective of HER-2 Levels. Molecular Medicine 8(8); 476-487 (2002).
36. **K.F. Pirollo**, A. Rait, L. Sleer, and E.H. CHANG. Antisense Therapeutics: From Theory to Clinical Practice. Pharmacology and Therapeutics 99: 55-77 (2003)
37. A.S. Rait, **K.F. Pirollo**, D. Ulick, K. Cullen and E.H. CHANG. HER-2 Targeted Antisense Oligonucleotide Results in Sensitization of Head and Neck Cancer Cells to Chemotherapeutic Agents. Ann. N.Y. Acad.Sci. 1002:78-89 (2003).

38. W. Yu, **K.F. Pirollo**, B. Yu, A. Rait, L Xiang, W.Q. Huang, Q. Zhou, G. Ertem, and E.H. CHANG. Enhanced transfection efficiency of a systemically delivered tumor-targeting immunolipoplex by inclusion of a pH-sensitive histidylated oligolysine peptide. Nucleic Acids Research 32 (5) e48 (2004).
39. W. Yu, **K.F. Pirollo**, A. Rait, B. Yu, L.M. Xiang, W.Q. Huang, Q. Zhou, G. Ertem, and E.H. CHANG. A sterically stabilized immunolipoplex for systemic administration of a therapeutic gene. Gene Therapy 11. 1434-1440 (2004)
40. **K. F. Pirollo**, G. Zon, A. Rait, Q. Zhou, W. Yu, R.Hogrefe, and E.H. CHANG. Tumor Targeting Nanoimmunoliposome Complex For Short Interfering RNA Delivery. Human Gene Therapy 17:117-124 (2006)
41. **K.F. Pirollo**, J. Dagata, P. Wang, M. Freedman, A. Vladar, S. Fricke, L. Ileva, Q. Zhou, and E.H. CHANG. A Tumor-targeted Nanodelivery System to Improve Early MRI Detection of Cancer. Molecular Imaging. In Press. (2006)
42. T. Beck, R. I. Hogrefe, A. V. Lebedev, **K. F. Pirollo**, A. Rait, Q. Zhou, W. Yu, E.H. CHANG, and G. Zon Chemically Modified Short Interfering Hybrids (siHybrids): Nanoimmunoliposome Delivery In Vitro and In Vivo for RNAi of HER-2 Submitted to Nucleic Acids Research.

## 3. BOOK CHAPTERS

1. **PIROLLO, K.F.**., Lin, X.Y., Hao, Z.M., Villegas, Z., and Chang, E.H. Molecular Mechanisms of Cellular Radioresistance and Radiosensitivity. In: "Radiation and the Gastrointestinal Tract". (A. Dubois, G.L. King, and D.R. Livengood, eds.) CRC Press, pp. 129-147 (1995).
2. Chang, E.H., Xu, L., and **PIROLLO, K.F.** Targeted p53 Gene Therapy Mediated Radiosensitization and Chemosensitization. Gutking, J.S., ed. The Human Press Inc. pp521-538, 1999.
3. **PIROLLO, K.F.**, Xu, L., and Chang, E.H. Immunoliposomes: A Targeted Delivery Tool for Cancer Treatment. In: "Vector Targeting for Therapeutic Gene Delivery". (D.T. Curiel and J.T. Douglas, eds.) John Wiley & Sons (In Press).

## 4. PROCEEDINGS

1. Srivastava, S., Zou, Z.Q., **PIROLLO, K.F.**., Tong, D., Sykes, V., Devadas, K., Miao, J., Chen, Y., Blattner, W., and Chang, E.H. In: Neoplastic Transformation in Human Cell System. Ed. J. Rhim (Humana Press), 125-134, 1991.
2. Freedman, M., Sarcone, A., **PIROLLO, K.F.**, Lin, C-S., and Chang, E.H. Ultrasound Images of Implanted Tumors in Nude Mice Using Sono-CT® Correlated with MRI Appearance. Proceedings of SPIE 5: 163-167 (2001).

## PATENT - APPLICATION FILED

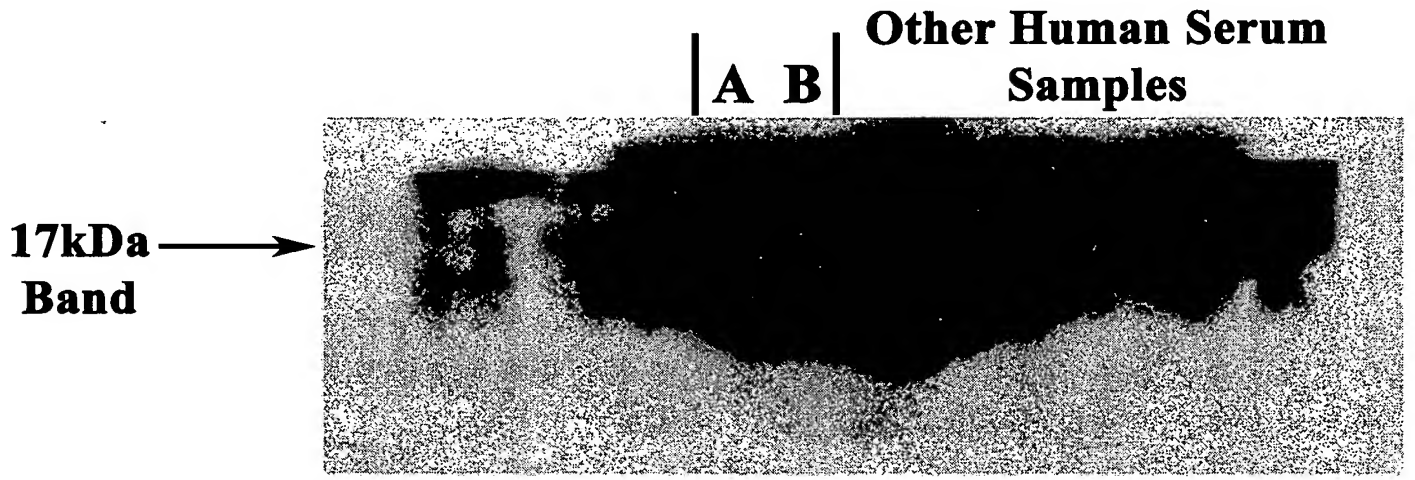
1. c-Raf Transgenic Non-Human Mammals.
2. Method for Reversal of Resistance to Radiation Therapy and to Chemotherapy in Cancer Cells Using Sequence Specific Anti-HER-2 Oligonucleotides. –Issued Feb. 22, 2000
3. Targeted Liposome Gene Delivery
4. Systemic Viral/Ligand Gene Delivery System
5. Compositions and Methods for Reducing Radiation and Drug resistance in Cells

6. A simplified and Improved Method for Complexing an Antibody Fragment Targeted Immunoliposome for Systemic Gene Therapy.

# EXHIBIT B

# Pre-Purification

A




# Post-Purification

B